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PRINCIPAL INVESTIGATOR: Sophie A. Lelièvre, D.V.M., Ph.D.

CONTRACTING ORGANIZATION:

University of California at Berkeley
E.O. Lawrence Berkeley National Laboratory
Berkeley, California 94720

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6. AUTHOR(S)
Sophie A. Lelièvre, D.V.M., Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of California at Berkeley
E.O. Lawrence Berkeley National Laboratory
Berkeley, California 94720

E-Mail: lelievre@purdue.edu

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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)* Matrix protease-mediated degradation of the basement membrane (BM) surrounding breast epithelial units (acini) is associated with tumor progression. It is critical to understand the molecular mechanisms that underlie the maintenance of an intact BM in order to develop anti-cancer strategies. Using a nonmalignant human breast epithelial cell line (S1) that differentiate into acini in the presence of extracellular matrix, we have identified earlier a link between the nuclear organization of the protein NuMA, via its C-terminus, and cell phenotype, notably matrix protease expression. We have expressed and purified the NuMA C-terminal histone-fold peptide that may be involved in the regulation of matrix proteases. Using this sequence as bait we have pulled down a 65 kDa ligand in nonmalignant cells but not in tumor cells. The histone-fold sequence has also been expressed in S1 cells and the resulting phenotype is being analyzed. We have identified a CH-actin binding domain at the N-terminus of NuMA that may be responsible for the protein anchorage to the cytoskeleton. We have also demonstrated that NuMA shuttles between nucleus and cytoplasm. Altogether our data suggest that NuMA may regulate cell phenotype not only by binding different c-terminus ligands but also by traveling between nucleus and cytoplasm.

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INTRODUCTION

Differentiation of functional and structural epithelial units of breast tissue, referred to as acini and ducts, is maintained by the presence of a continuous basement membrane. The breakdown of the basement membrane by matrix proteases has been associated with loss of structural and functional differentiation and tumor progression, as illustrated by the transition between ductal carcinoma in situ and invasive carcinoma. Understanding how an intact basement membrane is maintained in normal epithelial structures should help develop new therapeutic tools to prevent cancer progression. We have recently demonstrated a link between the organization of the nuclear mitotic apparatus protein, NuMA, and the activation of matrix proteases and subsequent degradation of the basement membrane [Lelièvre et al., 1998, Lelièvre et al., submitted]. We have hypothesized that NuMA organization induced by the formation of functional epithelial units is contingent upon its binding to other proteins to form multicomplexes, and that, in turn, the supramolecular organization of these multicomplexes is critical for the maintenance of the differentiation state. In order to decipher the molecular mechanisms that link NuMA organization to the maintenance of an intact basement membrane in breast epithelial units we have proposed to (1) investigate the role of specific N-terminal and C-terminal NuMA sequences we have identified as potential mediators of NuMA's functions (including matrix proteases activation) and (2) identify the binding partners of these sequences in conditions in which breast epithelial cells are and aren't differentiated into functional and structural units.

BODY

Our working model is a nonmalignant human mammary epithelial cell line HMT-3522 (S1) that can be induced to form functional acini surrounded by a complete endogenous basement membrane when cultured in the presence of an exogenous extracellular matrix (Matrigel^{BD}) for 10 days [Petersen et al., 1992]. Using this system we started to ***investigate the role of NuMA sequences identified as potential effectors of NuMA functions in the establishment and maintenance of an endogenous basement membrane (statement of work-task 1) and search for the binding partners of these sequences (statement of work-task 2)***.

In the first year report we showed that we had identified a putative histone-fold at the very C-terminus of NuMA and made FLAG-tagged c-DNA constructs for this sequence with and without the nuclear localization sequence (NLS) found at the beginning the histone-fold region. We had transfected the construct without the NLS [NuMA-CT-**no**NLS] into S1 cells and observed that it seemed located to the cytoplasm and did not have any significant effect on the differentiation process, as expected. Thus the expression of this construct was considered as an important negative control. Co-immunoprecipation experiments with an anti-FLAG antibody showed that a 95 kDa band was pulled down with NuMA-CT-**no**NLS. We had also commented on the progress of the preparation of other constructs, notably for the expression of NuMA histone-fold in bacteria. For the second year of the project we present results obtained from the expression of NuMA-histone fold sequence containing the NLS [NuMA-CT-**with**NLS] in S1 cells (task 1) and the preliminary results on the binding of NuMA-histone fold to different ligands in nonmalignant S1 and S1-derived malignant T4-2 breast epithelial cells (task 2). We also present new developments regarding the potential function of the N-terminus of NuMA and the way NuMA protein may coordinate BM signaling and nuclear structure. Based on the evolution of our research work we wish to propose an amended Statement of Work (sent as a separate document, along with the report).

***Task 2 (70% done)*: Search for NuMA binding partners. Year 2.**

Our preliminary results showed that antibodies directed against the C-terminus of NuMA could induce the reorganization of NuMA from nuclear peripheral domains to a diffuse distribution throughout the nucleus, mimicking a pattern found in tumor cells [Lelièvre et al., 1998]. This reorganization was sufficient to trigger matrix metalloprotease activity and degradation of the basement membrane. NuMA protein consists of N-terminal globular (residues 1-207) and C-terminal (residues 1729-2115) domains separated by a discontinuous 1500 amino acid coiled-coil region (Figure 1). In collaboration with Dr. Saira Mian from the Lawrence Berkeley National Laboratory (Berkeley, CA) we have identified a histone-fold sequence at the C-terminus of NuMA (base pairs 6203-6450 corresponding to amino-acids 1984 to 2064) that also includes the bipartite nuclear localization signal-NLS (between base pairs 6203 and 6260) [Gueth-Hallonet et al., 1996] (figure 2). This histone-fold sequence belongs to the part of the protein that is cleaved in response to the loss of cell-basement membrane interaction [Lelièvre et al., submitted] and is absent in the fusion protein formed by NuMA and the retinoic acid receptor that is thought to be responsible for the tumorigenic phenotype in certain leukemia [Wells et al., 1997] (figure 3A). Therefore the C-terminus of NuMA may play a critical role in the switch between normal and tumor behaviors. Our hypothesis is that the C-terminus of NuMA binds to different partners in nonmalignant and tumor cells. To test this hypothesis, we have engineered two subsets of cDNA including the putative histone-fold domain by PCR: One contains the NLS and is based on the utilization of primers 1 and 3 (figure 3B), the other one is based on the utilization of primers 2 and 3 and is devoid of NLS sequence (figure 3B). The latter construct was intended to be used as a negative control for the nuclear effects of the C-terminal sequence transfected into nonmalignant breast epithelial cells, since it shouldn't translocate to the cell nucleus, while construct 1-3 should. Results presented in the year 1 report with the construct devoid of NLS confirmed this assumption.

Figure 1.

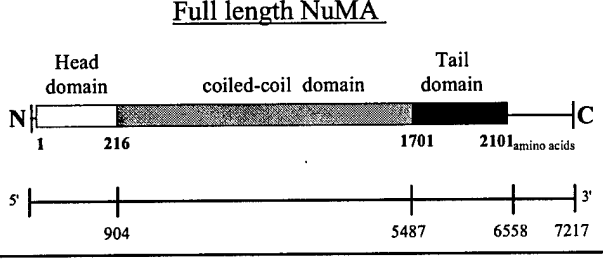


Figure 2.

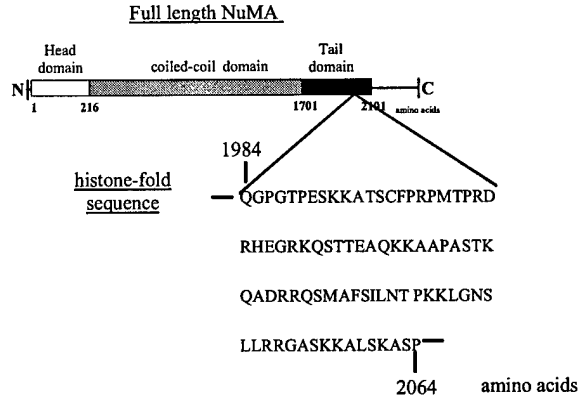
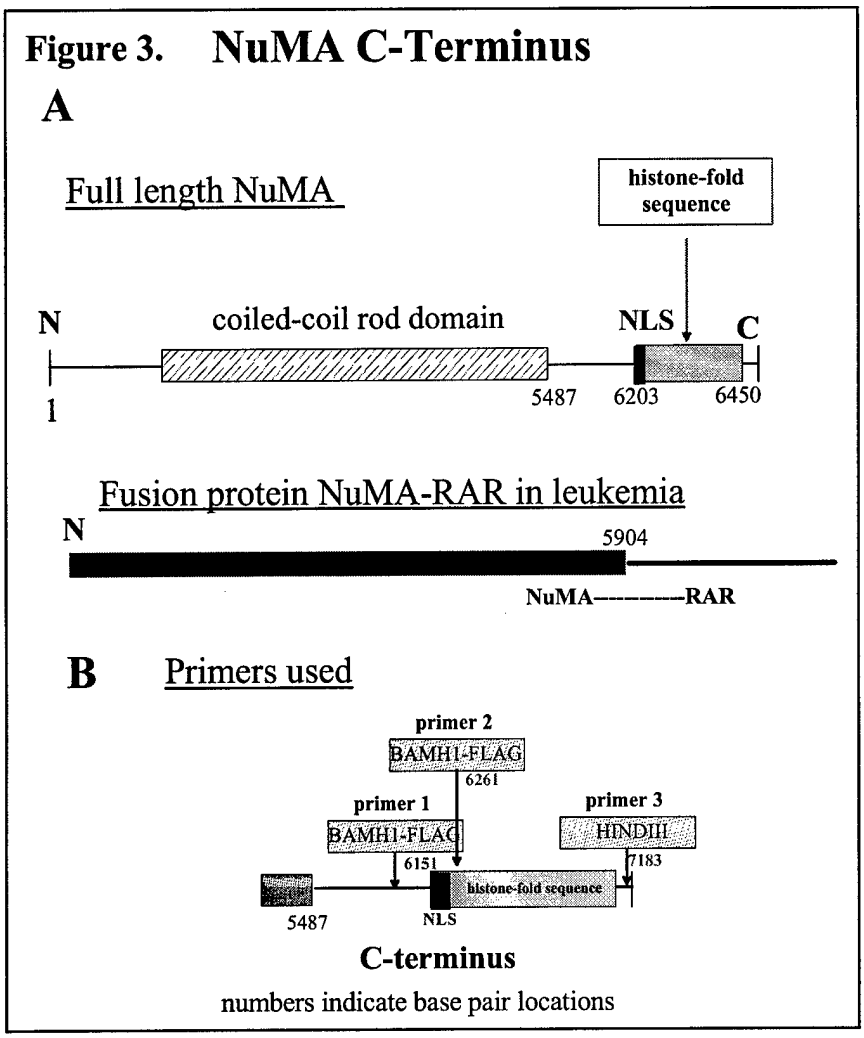
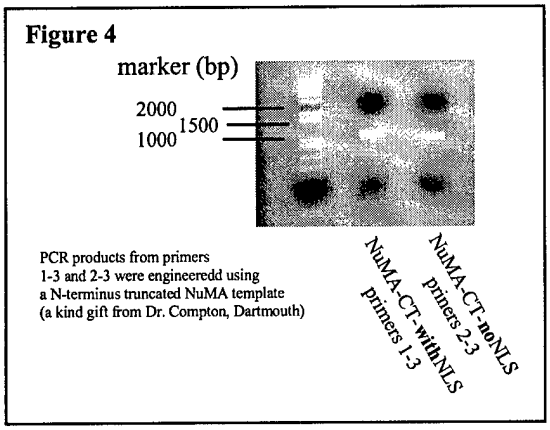


Figure 3. NuMA C-Terminus



The preparation of FLAG-tagged NuMA C-terminal constructs (corresponding to the use of primers 1-3 and 2-3) was described in last year's report (figure 4). In order to detect potential ligands of NuMA-histone fold we have expressed and purified the corresponding peptide *in vitro* as a poly-his fusion protein and used it as bait in affinity binding assay and Far Western blot experiments. More specifically, we have subcloned the cDNA for NuMA-CT**with**NLS (NLS + full histone-fold sequence) in pRSET-A vector plasmid that contains a polyhistidine (6X) coding DNA sequence upstream of the cloning site. The choice of 6X His, instead of GST, for the fusion protein was based on the fact that our NuMA sequence is very small and GST is a big peptide that could impair the function and/or protein binding of NuMA-CT; whereas 6X His has been reported to allow complete function of small peptides. Polyhis-FLAG-NuMA-CT**with**NLS expression was induced in one-liter cultures of E. Coli (BL21-DE3-pLys) with 1 mM IPTG for five hours (longer induction time resulted in a decrease in bacterial population probably due to a toxic effect associated with the expression of NuMA construct). The fusion peptide was purified in its native form using metal-exchange column (Xpress system from Invitrogen) and pH-based elution. Eluted fractions were run on 15% polyacrylamide gel, transferred onto a nitrocellulose membrane and immunoblotted with anti-FLAG antibody. Five bands were detected, at 18, 20, 25, 34 and 36 kDa (figure 5A). These molecular weights were established from the standard calibration curve drawn from the migration position of standard molecular weight markers run

on the same gel. The correct size for poly-his NuMA-histone-fold is 18 kDa, as determined from the c-DNA sequence. The 34 kDa turned out to be a protein to which the secondary antibody binds nonspecifically, while the 36 and 25kDa bands were also recognized nonspecifically in preparations made from BL21-DE3-pLys bacteria transfected with empty RSET-A vector (figure 5B). The 20kDa band is from unknown origin. These results show that 18kDa NuMA-histone-fold can be successfully expressed and purified in a native form from bacteria.



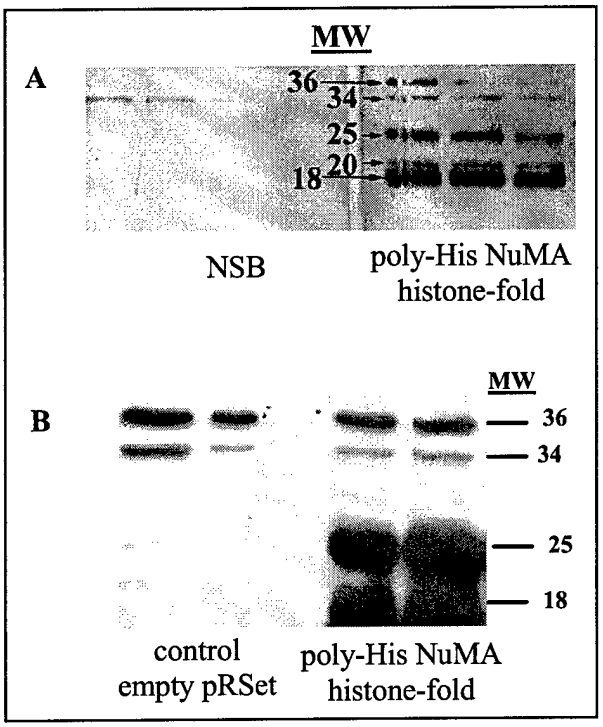


Figure 5. Purification of 18 kDa poly-His NuMA histone-fold. A: blotting of poly-His FLAG-NuMA histone-fold elution fraction from a Nickel column with horseradish peroxidase conjugated secondary antibody only shows a nonspecific binding at 34 kDa (NSB). Blotting with primary antibody anti-FLAG followed by horseradish peroxidase conjugated secondary antibody shows 5 bands at 36, 34, 25, 20 and 18 kDa (poly-His NuMA histone-fold).

B: Nickel column purification of protein extracts from bacteria transfected with an empty pRSet plasmid (control empty pRSet) shows that 36, 34 and 25 kDa bands seen in poly-His NuMA fractions are nonspecific. Only the 18 kDa band remains specific of the elution fraction of poly-His FLAG-NuMA histone-fold. The primary antibody used in western blot analysis is anti-FLAG. (MW = Molecular weight).

Elution fractions containing poly-his NuMA-histone-fold and control fractions from empty vector-transfected bacteria were used in affinity binding assays. Elution fractions were

dialyzed in 50 mM Tris pH 7.9, 500 mM NaCl, and sodium azide, while RIPA extracts from nonmalignant S1 cells and S1-derived malignant T4-2 cells were dialyzed in 50 mM Tris pH 7.9, 150 mM NaCl, 1 mM MgCl2, 10% glycerol and 1 mM PMSF. Five micrograms of dialyzed poly-his NuMA fusion peptide were mixed with 100 µl of Ni^{2+}-NTA beads slurry. After binding, NuMA peptide-beads were washed in buffers with decreasing NaCl concentrations. Purified extracts from the control bacteria containing the empty plasmid were subjected to the same treatment to visualize a baseline for undesired proteins. 25 µg of protein extracts from nonmalignant S1 and malignant T4-2 cells were incubated for two hours with the NuMA peptide-bead preparation. Elution of NuMA binding partners was performed with 100 µl of 500 mM NaCl containing buffer. Finally beads were incubated with 500 mM imidazole containing buffer to remove any protein bound to the nickel beads, including poly-His NuMA peptide. Half of the fractions were run on a polyacrylamide gel. Proteins were transferred onto nitrocellulose and the presence of NuMA-histone fold in the elution fractions was assessed using the anti-Flag antibody (Figure 6). NuMA peptide was only detected in fractions supposed to contain this peptide. The other half of the fractions were run on a polyacrylamide gel and the gel was silver stained. A 18kDa band was found in the fractions that were supposed to contain NuMA peptide. In addition one band at 65kDA was found only in protein extracts from S1 cells, but not in T4-2 cells or in controls used for the detection of non specific binding (figure 7). This experiment will be repeated several times during the third year of the research project.

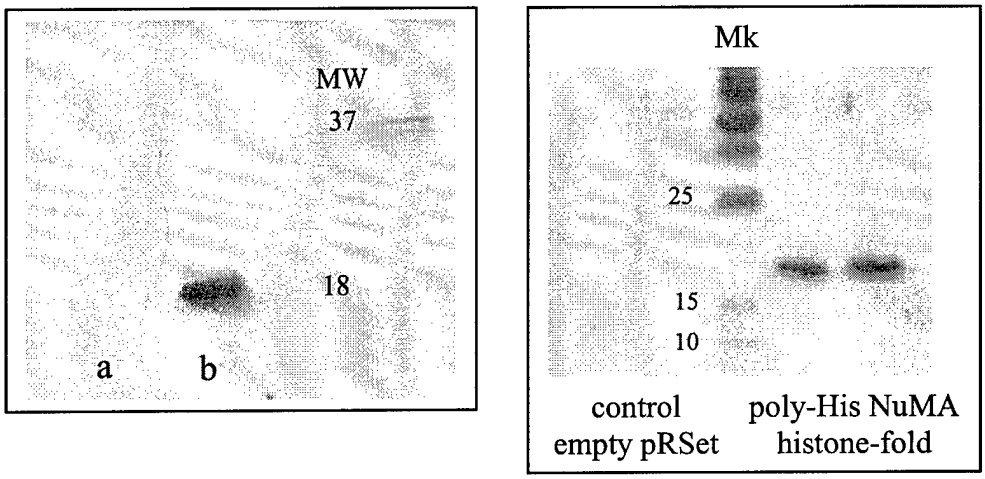


Figure 6. Left panel: Loading of Nickel-NTA beads. After incubation of the empty vector fraction (a) and poly-his NuMA histone-fold fraction (b) with the nickel beads slurry, a fraction of the beads was run on a 15% gel and proteins were transferred onto a nitrocellulose membrane. Western blot analysis with anti-FLAG antibody shows that only the beads incubated with the poly-His NuMA fraction were coated with an 18 kDa peptide (which corresponds to the molecular weight of poly-His FLAG-NuMA histone-fold).
Right Panel: elution of poly-His NuMA from the beads at the end of the affinity binding assay. After incubation with the protein extracts from S1 and T4-2 cells, and elution of the ligands with 500 mM NaCl, the bead slurry was incubated with 500 mM imidazole to detach proteins bound to Nickel. Only the beads previously incubated with poly-His FLAG-NuMA histone fold show a 18 kDa band on the anti- FLAG western blot. (Mk = molecular weight marker)

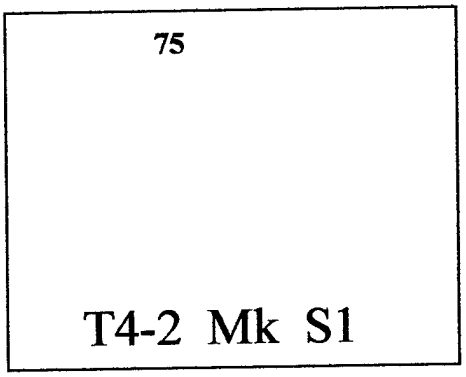


Figure 7. Silver staining of elution fractions from affinity binding assay. Protein extracts from nonmalignant (S1) and malignant (T4-2) cells were incubated with NuMA histone-fold coated beads. NuMA ligands were then eluted with 100 µl of elution buffer containing 500 mM NaCl. Elution fractions were run on a 7.5% gel and the gel was silver stained (Mk =marker). A band is seen below the 75 kDa marker in the S1 lane but not in the T4-2 lane.

Far Western blot experiments were also performed to detect NuMA-histone fold ligands as follows: 50 µg of protein extracts from nonmalignant S1 and malignant T4-2 cells were run on 10 % acrylamide gel. Proteins were transferred onto a nitrocellulose membrane and incubated with 25 µg of NuMA-peptide or preparation from empty vector control, followed by incubation with anti-FLAG antibody and horse-radish peroxidase conjugated secondary antibody. Preliminary results reveal a high background. One band around 110 kDA was detected in S1 cells but not in T4-2 cells or in controls for nonspecific binding (not shown). However, this result was not reproduced with the second set of experiments. In year three we will redo these experiments using double-purified NuMA peptide (by passing the peptide fraction twice on a nickel column to get rid of nonspecific proteins) and the incubation for far western blot will be done directly on proteins run in non-denaturing conditions. After the incubation steps the proteins that will have bound NuMA peptide will be directly detected on the gel with anti-FLAG and horseradish peroxidase-conjugated secondary antibody. We hope that by using non-denaturing conditions in the far western blot technique we may be able to confirm the NuMA-histone-fold binding to a 65kDa protein as detected with affinity binding assay (in which the binding to NuMA peptide occurred in non-denaturing conditions).

During the third year of the project we will also investigate possible 65kDa protein candidates. We will first look into other proteins that possess a histone-fold since this sequence is often found in proteins that form multi-protein complexes at the chromatin level.

Since the affinity biding assay and far western techniques described above give encouraging results, we will not attempt using the two-hybrid system. Indeed the two-hybrid system would not enable us to directly check for ligands that are specifically found in nonmalignant cells compared to nonmalignant cells.

Unexpected research direction: The assessment of the binding partners for the N-terminus of NuMA has led to surprising results.

Our original experiments using anti-NuMA antibodies to disrupt NuMA organization (Lelièvre et al., 1998), showed that these antibodies directly went to the cell nucleus after gentle digitonin permeabilization of the plasma membrane. Digitonin selectively permeabilizes cellular membranes rich in cholesterol, like the plasma membrane, while intracellular organelle membranes, including the nuclear envelope, with relatively low levels of cholesterol remain intact (Adam et al., 1990; Liu et al., 1999). Then, cells are fixed and stained with a fluorochrome-tagged secondary antibody that permits the visualization (by fluorescence) of the primary antibody incorporated upon digitonin permeabilization. Experiments done with antibodies specifically directed against the N-terminus of NuMA showed that these antibodies

first accumulated in the cell nucleus upon *in vivo* digitonin permeabilization of breast epithelial cells organized into acini, and then returned to the cytoplasm. Since antibodies do not cross the nuclear envelope on their own, this preliminary result led us to hypothesize that the antibodies might be translocating between cytoplasm and nucleus with their antigen, NuMA. As a control we incubated digitonin-permeabilized cells with immunoglobulins (mouse IgGs). Staining with an anti-mouse IgG secondary antibody only showed a diffuse pattern for IgG in the cytoplasm only. We also verified that the one-minute digitonin treatment did not induce cell death, using trypan blue dye exclusion test and apoptag assay, up to five days after treatment. At the time we were doing these experiments, we also started looking at NuMA distribution with electron microscopy. Surprisingly, electron micrographs revealed the presence of NuMA in both nucleus and cytoplasm. The fact that NuMA is not usually detected in the cytoplasm with low-resolution fluorescence indicates that it resides in this compartment in small amounts. The presence of NuMA in the cytoplasm was confirmed by soft-X-ray microscopy another high-resolution microscopy. Altogether these results suggested us that NuMA may be shuttling between nucleus and cytoplasm.

During the past two years we did two types of experiments critical to test the hypothesis of NuMA nucleo-cytoplasmic shuttling. First we repeated anti-NuMA antibody translocation experiments in the presence of cyclohexamide, an inhibitor of protein synthesis. The antibody still accumulated in the cell nucleus suggesting that antibody translocation was not associated with de novo synthesis of NuMA **(figure 8)**. We also used monolayer cultures of S1 cells to perform heterokaryon analysis, an assay considered to be the ultimate evidence for nucleocytoplasmic shuttling (Hache et al., 1999; Fischer et al., 1999). In this assay, human cells are fused to mouse cells following polyethylene glycol treatment, incubated with cyclohexamide, and fixed at different times for immunostaining (Hache et al., 1999). Immunostaining is performed with an antibody that only recognizes the human isoform of the protein to be tested. Thus, for a protein that usually shows nuclear steady state, if the staining is observed in both the murine nucleus and human nucleus of a fused cell, it indicates that the human isoform of the protein studied has traveled from the human nucleus to the cytoplasm of the heterokaryon and then into the murine nucleus. Murine nuclei are easily distinguished by the presence of bright chromatin aggregates as shown by DAPI staining. A few hours following heterokaryon formation between our human S1 cells and NIH-3T3 mouse cells, immunostaining performed with an antibody that only recognizes the human isoform of NuMA (clone 107.7.8, a kind gift from Dr. Nickerson, Umass) showed the presence of NuMA in both murine and human nuclei **(Figure 9)**, thus confirming NuMA nucleo-cytoplasmic shuttling.

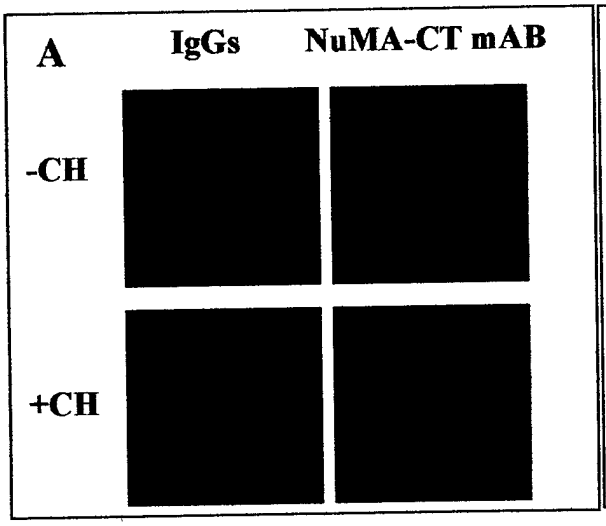


Figure 8. NuMA shuttles in differentiated cells in the presence of an inhibitor of protein translation. Nonmalignant S-1 cells were allowed to differentiate into acini by growing them in 3D culture for 10 days. Live acini were incubated without (-CH) or with (+CH) cyclohexamide for three hours to block protein translation, then permeabilized with 0.01% digitonin in permeabilization buffer and incubated with control immunoglobulins (IgGs) or antibodies against the C-terminus of NuMA (NuMA-CT mAb) in the absence (-CH) or presence (+CH) of cyclohexamide for 12 hours. After 12 hours, immunostaining with Texas Red-conjugated secondary antibody showed that the antibody had reached the nucleus in both the absence and presence of cyclohexamide, while IgGs remain cytoplasmic. These results indicate that NuMA antibody traveling to the cell nucleus is not associated with de novo synthesis of NuMA. All the images were obtained by confocal microscopy.

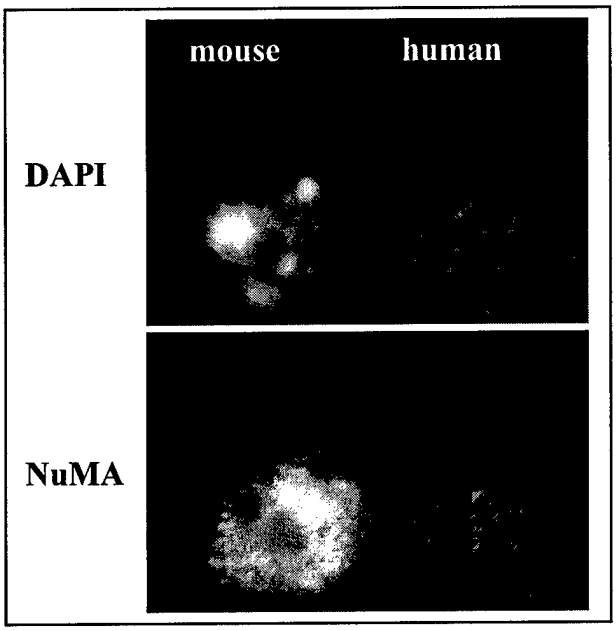
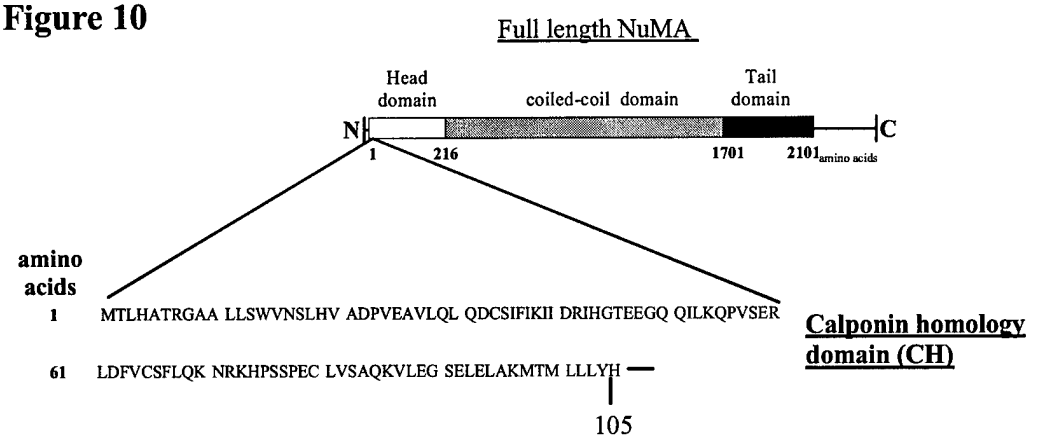


Figure 9. Heterokaryon analysis demonstrates that NuMA shuttles between nucleus and cytoplasm in breast epithelial cells grown as a monolayer (2D culture). Nonmalignant S1 cells and NIH-3T3 murine cells were co-cultured for 2 days, fused using polyethylene glycol and treated with cyclohexamide to prevent de novo protein synthesis. Cells were fixed and immunostained at different time intervals with an antibody that recognizes only the human isoform of NuMA (clone 107.7.8). A murine nucleus is identified by its bright speckles next to a human nucleus (DAPI, upper image panel A). NuMA staining in found in both the murine and human nuclei (NuMA lower image, panel A). NuMA staining could not be detected in isolated murine nuclei (control with non-fused cells, not shown).

When we started looking at the N-terminal sequence of NuMA for the DOD project, we identified a calponin homology (CH)-binding domain at the very beginning of NuMA sequence (**figure 10**). The CH domain is an actin-binding sequence through which a protein directly interacts with F-actin and/or bridges F-actin and intermediate filament networks (Hanein et al., 1997). This domain was identified by computational search of the database for putative binding motifs in NuMA (Mian and Lelièvre). The search methods used by our collaborator, Dr. Mian, were not common BLAST or PSI BLAST techniques but methods that search with a single sequence against a library of models for protein families. The CH-domain was found in the profile database at ISREC (Geneva). The CH domain was originally described at the N-terminus of calponin, an actin-binding protein that regulates smooth muscle contraction (Castresana and Saraste 1995). It is also present as a single copy in signaling proteins, including Vav and IQGAP (Castresana and Saraste 1995; Epp and Chant, 1997). Thus similarly to the SH3 domain, the CH domain represents another case of a protein module present both in cytoskeletal and signaling proteins. We prepared cell extracts to clearly visualize the cytoskeleton in EM and found NuMA significantly present on both actin filaments and intermediate filaments (**figure 11**). Based on these preliminary results we believe that the N-terminus of NuMA may serve as an anchor to the cell skeleton, at least when NuMA is in the cytoplasm. The possible anchorage of NuMA via its N-terminus is supported by the fact that although NuMA C-terminus can be easily immunoprecipitated after cleavage of the protein, it is extremely difficult to immunoprecipitate full length or C-terminus truncated NuMA. In addition, direct immunofluorescence with antibodies directed against the N-terminus of NuMA does not give any signals even after triton extraction, a technique used to unravel the epitopes of certain proteins. However, when introduced in live cells the same N-terminus directed antibodies can bind to shuttling NuMA and hence be revealed with secondary antibodies upon fixation. Finally, to the best of our knowledge, so far all NuMA ligands identified with techniques (e.g., the yeast to hybrid system or co-immunoprecipitation with cell extracts) that cannot test for interactions with cytoskeletal network are only found to bind the C-terminus of NuMA. ***Therefore instead of searching for potential ligand to NuMA N-terminus in a way similar to that done for NuMA C-terminus (see***

task 2), we need to specifically investigate NuMA binding to F-actin and intermediate filaments.

Figure 10



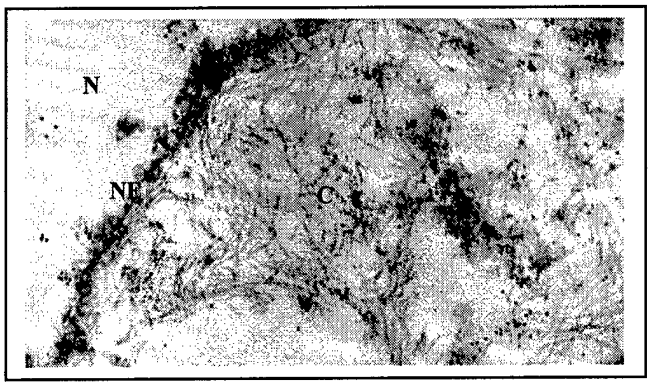


Figure 11. NuMA is localized to the cytoskeleton in nomalignant and malignant breast epithelial cells. Tumor T4-2 cells were induced to form tumors in 3D culture. After *in situ* nuclear matrix preparation that removes soluble proteins and DNA, cultures were sectioned, immunolabeled for NuMA and prepared for EM staining. NuMA is located to the cytoskeleton (compartment **c**) (see black 5 nm nanogold particles). (Magnification 36,000) N: nucleus; NE: nuclear envelope; C: cytoplasm.

Our current hypothesis is that the N-terminus of NuMA is involved in NuMA anchorage and may partly regulate NuMA shuttling, while the C-terminus of NuMA may be involved in the binding of ligands to be shuttled. Alternatively, The shuttling of NuMA may be associated with the posttranslational modification of certain parts of this protein (notably its C-terminus). These hypotheses will not be tested in the current project. During year 3 of our research project we will limit the work on the N-terminus of NuMA to the production of NuMA sequence lacking the N-terminus (see task 1) and transfection of S1 cells with this construct to test whether it affects metalloprotease activity, which is a major aim of this proposal.

Task 1 (50% done): **Production and analysis of acini expressing truncated forms of NuMA. Year 2.**

The purpose of this task is to transfect nonmalignant S1 cells with different truncated forms of NuMA in order to alter differentiation and possibly influence metalloprotease activity. Since the C-terminus of NuMA seems to play a critical role in the regulation of acini differentiation and metalloprotease activity, we expect that either the histone-binding sequence

of NuMA or NuMA truncated for its C-terminus may induce a dominant negative phenotype (e.g., lack of differentiation, activation of metalloproteases).

NuMA-histone-fold inserts were prepared for cell transfection by subcloning into pcDNA 3.1 plasmids and selecting for ampicillin resistance. Non-malignant S1-cells were transfected with the NuMA putative histone-fold sequence containing the NLS [NuMA-CT-**with**NLS] and selected for neomycin resistance with G418. Selected transfectants were subcloned using the ring method and 20 clones were tested for the expression of the transgene. Expression of NuMA-CT-**with**NLS in S1 cells was assessed by western blot analysis of total protein extracts (Leammli procedure) ran within a 15% polyacrylamide gel and transferred onto a nitrocellulose membrane. Immunoblotting with anti-FLAG antibody revealed a band around 18 kDA in about 2/3 of the clones. No such band was seen in cells transfected with control empty vector (**figure 12**). We did not see for this sequence bands that may correspond to the dimerization of NuMA peptide as we had seen with the sequence without NLS expressed in S1 cells or in bacteria. [We had proposed in last year's report to test whether this higher molecular weight band was due to dimerization of NuMA C-terminus by treating the extracts with urea before electrophoresis. We did that experiment and could not get rid of the higher molecular weight bands. Therefore we still do not know the origin of these extra-bands obtained with the construct without NLS].

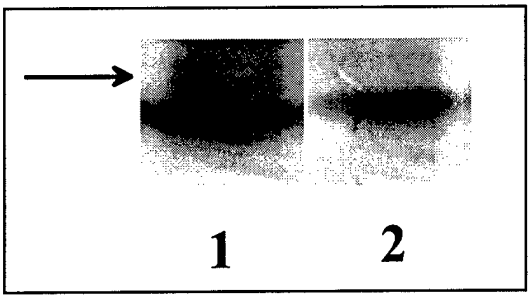


Figure 12. Expression of NuMA histone-fold in S1 cells. Total protein extracts were prepared from S1 cells transfected with FLAG-tagged NuMA histone-fold (1) or pcDNA empty vertor (2). Proteins were run on a 15% polyacrylamide gel and transferred onto a nitrocellulose membrane. Western blot with anti-FLAG antibody shows a faint band around 18kDa (see arrows) in S1 transfected with NuMA histone fold.

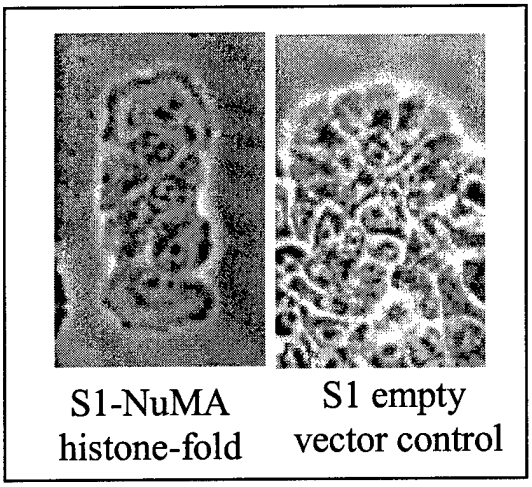
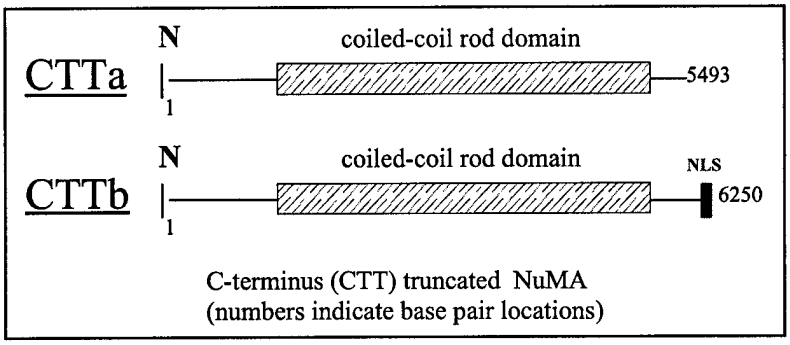


Figure 13. Phase contrast images of 2D cultures of S1 cells transfected with NuMA histone fold (left image) and empty vector control (right image). S1 cells tranfected with NuMA histone-fold show change in cell shape ("wavy" cell membrane).

When cultured as a monolayer, some of the NuMA-CT-**with**NLS S1 transfectants show an altered cell shape compared to empty control vector transfected cells and regular S1 cells (**figure 13**). Preliminary results also indicate a different behavior in 3D culture (under analysis). Therefore the expression of NuMA histone-fold containing the NLS may give a phenotype different from that of NuMA histone-fold without the NLS, which lacks the first part of the histone-fold sequence (NuMA-CT-**no**NLS) [the latter showed no detectable phenotype in both

monolayer and 3D culture, see year 1 report]. We have previously shown that antibodies directed against the C-terminus of NuMA and introduced into the nucleus of acinar S1 cells induce metalloprotease activation and subsequent basement membrane degradation as well as apoptosis, although via independent mechanisms. We are now in the process of assessing basement membrane integrity and the level of apoptosis in the transfectants. 3D cultures of the different clones have been frozen and will be used during year 3 for immunostaining on cryosections. The localization of exogenous FLAG-tagged NuMA peptide will also be assessed. Then clones showing degradation of the basement membrane and/or apotosis will be thawed and tested for metalloprotease activity and invasion capabilities. (year 3). To confirm the phenotype, more transfections will be established with constructs bearing a better tag than FLAG for immunostaining. In future transfections, we envision using V5-a 14 amino acid epitope, as a tag. It has been shown to work well for immunolocalization in the laboratory of our consultant Dr. Judy Campisi.

Another section of task 2 was to express NuMA lacking the C-terminus (CCTa, **figure 14**) under inducible TET system. Since the viral TET system had not given satisfaction to our collaborator and consultant in their own studies, we went back to the original transfection-based system. A response plasmid pUDH 10-3 including the tet operator and a regulator plasmid pUHD15-1 including the tTA gene was made available to us by our colleague Dr Andrisani (Purdue University); S1 cells were transfected with tTa construct and selected with G418 in three different experiments. Unfortunately no clone was found acceptable (e.g, very slow growth and ultimately growth arrest at very low cell density). Since the behavior of our cells is critical for our experiments, we decided not to pursue the use of the TET system, especially since a double transfection and selection is ultimately necessary to get expression of the peptide of interest, therefore lessening the chance of getting healthy cells before even starting a specific study. Instead, during year 3, we will directly transfect truncated NuMA contructs in our S1 cells. This construct will code for NuMA up the NLS sequence (CTTb, **figure 14**); thus 90% of the histone-fold will be missing. We prefer to use this construct at this point rather than a construct that would lack the entire c-terminus of NuMA (which we proposed to use originally), since it will be a direct complementary construct to NuMA-histone fold and hence may act as a dominant negative for the function of this particular sequence. The construct will be inserted into the pCDNA-V5 plasmid. S1 cells will be transfected as monolayers, selected for G418 and subcloned. Clones will then be plated in 3D culture. The resulting phenotype will be tested. In order to get rid of any effect due to the interaction of the exogenous protein with cell proliferation (NuMA has been involved in mitotic spindle organization), we will also directly transfect our differentiated acini, with the same construct, in 3D culture by electroporation. This technique has been successfully used to transfect differentiated cells *in vivo* [Osumi and Inoue, 2001; Pucihar et al., 2001; Weaver and Hogan, 2001]. We have recently purchased a biorad electroporator and our colleague at Purdue, Dr. Hannon, has been using tissue electroporation for the past 18 months and will assist us in the establishment of this technique in the laboratory. The construct lacking the N-terminus of NuMA (see task 2) will also be transfected in S1 cells using these methods.



TIMETABLE (according to current statement of work)

Task 1A	months 1-6: in progress (50%)
Task 1B	months 6-10: in progress (40%)
Task 1C	months 9-15: to be initiated
Task 2A	months 1-6: complete
Task 2B	months 6-36: replaced by task 2D
Task 2C	months 15-21: complete
Task 2D	months 21-36: 60% (in progress)

KEY RESEARCH ACCOMPLISHMENTS-year 2.

- Nonmalignant S1 breast epithelial cells transfected with NuMA histone-fold sequence including the NLS [NuMA-CT-withNLS] seem to have altered their differentiation capability.
- NuMA histone-fold sequence [NuMA-CT-withNLS] was expressed in bacteria and purified as a native poly-his fusion peptide.
- Affinity binding assay with poly-his NuMA histone-fold indicates the presence of a ligand for this sequence in nonmalignant cells; this ligand is not found in the malignant counterpart.
- NuMA is capable of shuttling between nucleus and cytoplasm (combination year 1 and 2)
- NuMA N-terminus contains a CH-actin binding domain that may be responsible for NuMA anchorage to the cytoskeleton.

REPORTABLE OUTCOMES- year 1.

1) Development of cell lines: nonmalignant S1 cells expressing a potential histone-fold sequence in NuMA C-terminus.
2) Production of purified poly-His-fusion peptide for the putative histone-fold sequence of NuMA.
3) Patricia Abad, the research assistant working on the project has been promoted to research professional (AP) level. She has also been accepted into graduate school and is working as a part time student on her master's thesis. For her work with NuMA, she recently received honorable mention at a university wide poster presentation.
4) Training: Zoltan Metlagel, a senior undergraduate student, who worked on the bacterial expression of poly-his NuMA peptide and affinity binding assay received a summer fellowship (2002) from the Caroll County Cancer association.
5) Sophie Lelièvre presented some of the NuMA work as part of her research program presentation in front of an external review panel gathered to evaluate the status of Walther Cancer Institute (WCI) investigators. Her WCI status is maintained.
6) An RO-1 grant application was sent to NIH to continue the work on NuMA shuttling (status pending).
7) A predoctoral fellowship application based on the NuMA work was submitted to DOD by Patricia Abad as part of her future status of full time PhD student (starting in the Fall of 2003) (status pending)

8) A US patent (# 6,287,790 B1) based on work previously supported by DOD (to Sophie A. Lelièvre) was issued in September 2001. The patent deals mostly with the use of NuMA distribution as a marker of cancer progression.
9) A manuscript containing some of the data presented in the report is in preparation (P Abad, A Viron, JA Nickerson, E Puvion, and SA Lelièvre. The nuclear mitotic apparatus protein, NuMA, shuttles between nucleus and cytoplasm in breast epithelial cells differentiated into glandular structures).
10) Five abstracts containing some of the data presented in the report were presented/submitted at local (Phi Zeta day, Sigma Xi day), national (Era of Hope) and international (ASCB, 2nd International conference on tumor microenvironment) scientific meetings.

CONCLUSIONS

During the second year of the research project, we have expressed the cDNA corresponding to a putative histone-fold domain (containing the NLS) found at the C-terminus of NuMA in nonmalignant S1 cells. Preliminary results show that these cells may have an altered phenotype. We have also expressed NuMA histone-fold as a poly-His fusion peptide and used it as bait to pull down potential NuMA binding partners. A 65 kDa ligand has been observed in nonmalignant cells but not in malignant cells.

A putative CH-actin binding domain has been identified at the N-terminus of NuMA. This sequence may be responsible for NuMA anchorage to the cytoskeleton. We have also demonstrated that NuMA is shuttling between nucleus and cytoplasm. This result is quite important since it may explain how NuMA may coordinate basement membrane signaling and nuclear events.

During year 3 of the research project, emphasis will be put on NuMA histone-fold binding partners, the expression of NuMA truncated at its C-terminus, and the detailed analysis of the phenotype of S1 cells transfected with NuMA histone-fold (already available) and C-terminus truncated NuMA (to be prepared), especially with regards to the regulation of metalloprotease activity (a major goal of the DOD project). The work with the N-terminus will not be pursued further in this proposal since our preliminary results indicate it pertains to a totally new direction that would go beyond the purpose and the time frame of the DOD project. Hence, the work regarding the potential interaction between NuMA N-terminus and the cytoskeleton will be continued in another project. However, if time permits we will express NuMA N-terminus truncated in S1 cells to see if it has an effect on metalloprotease activity and if this exogenous truncated protein can still bind the 65kDa protein that binds NuMA C-teminus (see this report) , using co-immunoprecipitation.

REFERENCES

Lelièvre, S. A., V. M. Weaver, et al. (1998). "Tissue phenotype depends on reciprocal interactions between the extracellular matrix and the structural organization of the nucleus." *Proc Natl Acad Sci U S A* **95**(25): 14711-6.

Petersen, O. W., L. Ronnov-Jessen, et al. (1992). "Interaction with basement membrane

serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells." *Proc Natl Acad Sci U S A* **89**(19): 9064-8.

Gueth-Hallonet C, Weber K, Osborn M. (1996). NuMA: a bipartite nuclear location signal and other functional properties of the tail domains. *Exp Cell Res.*, 225:207-18

Wells RA, Catzavelos C, Kamel-Reid S. (1997) Fusion of retinoic acid receptor alpha to NuMA, the nuclear mitotic apparatus protein, by a variant translocation in acute promyelocytic leukaemia. *Nat Genet.* 7(1): 109-13.

Adam, S.A., R.S. Marr, and L. Gerace. 1990. Nuclear protein import in permeabilized mammalian cells requires soluble cytoplasmic factors. *J Cell Biol.* 111:807-16.

Liu, J., N. Xiao, and D.B. DeFranco. 1999. Use of digitonin-permeabilized cells in studies of steroid receptor subnuclear trafficking. *Methods.* 19:403-9.

Hache, R.J., R. Tse, T. Reich, J.G. Savory, and Y.A. Lefebvre. 1999. Nucleocytoplasmic trafficking of steroid-free glucocorticoid receptor. *J Biol Chem.* 274:1432-9.

Fischer, N., M.D. Voss, N. Mueller-Lantzsch, and F.A. Grasser. 1999. A potential NES of the Epstein-Barr virus nuclear antigen 1 (EBNA1) does not confer shuttling. *FEBS Lett.* 447:311-4.

Hanein, D., P. Matsudaira, and D.J. DeRosier. 1997. Evidence for a conformational change in actin induced by fimbrin (N375) binding. *J Cell Biol.* 139:387-96.

Castresana, J., and M. Saraste. 1995. Does Vav bind to F-actin through a CH domain? *FEBS Lett.* 374:149-51.

Epp, J.A., and J. Chant. 1997. An IQGAP-related protein controls actin-ring formation and cytokinesis in yeast. *Curr Biol.* 7:921-9.

Osumi, N., and T. Inoue. 2001. Gene transfer into cultured mammalian embryos by electroporation. *Methods.* 24:35-42.

Pucihar, G., T. Kotnik, M. Kanduser, and D. Miklavcic. 2001. The influence of medium conductivity on electropermeabilization and survival of cells in vitro. *Bioelectrochemistry.* 54:107-15.

Weaver, M., and B. Hogan. 2001. Powerful ideas driven by simple tools: lessons from experimental embryology. *Nat Cell Biol.* 3:E165-7.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Sophie A. Lelièvre	Walther Assistant Professor of Basic Medical Sciences

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Louvain, Belgium	Engineer	1984-1987	Veterinary Sciences
University of Liège, Belgium	Medical Degree	1987-1990	Veterinary Medicine
University of Paris VI, France	Master's	1990-1991	Molecular and Cell Pharmacology
University of Paris VI, France	Ph.D.	1991-1994	Molecular and Cell Pharmacology
Lawrence Berkeley Natl Lab, Berkeley, USA	postdoc	1995-1999	Mammary Cell Biology

RESEARCH AND PROFESSIONAL EXPERIENCE

1991-1995: **Veterinary Surgeon**	Pets Emergency Room, Paris District.
1991-1994: **Predoctoral Fellow**	Gustave Roussy Cancer Institute (Villejuif, France); topics: topoisomerases, anti-cancer pharmacology.
1991-1994: **Teaching assistant**	University of Paris; topics: Embryology and Histology.
1995: **Postdoctoral Fellow**	Gustave Roussy Cancer Institute (Villejuif, France), and a 3-month training in Dr Kohwi-Shigematsu's laboratory (LJCRF, La Jolla, CA); topics: resistance to topoisomerase inhibitors, metastatic phenotype, M.A.R., nuclear matrix.
1995-1997: **Postdoctoral Fellow**	Lawrence Berkeley National Laboratory; Dr Mina Bissell's laboratory (Berkeley, CA);
1997-1999: **Postdoctoral Scientist**	topics: extracellular matrix-nuclear structure interrelationship, regulation of gene expression in breast morphogenesis and tumorigenesis.
1999-2000: **Research Scientist**	Cell and Molecular Biology Dept. Lawrence Berkeley National Lab., Berkeley CA; Topics: nuclear organization and gene expression, nuclear signaling.
2000-ongoing: **Assistant Professor and Walther Investigator**	Basic Medical Sciences Dept., Purdue University, West Lafayette IN. Topics: nuclear organization in differentiation and cancer, nuclear signaling, nuclear structure and genomic instability.

HONORS

University of Louvain (Belgium), lifetime tuition exemption for outstanding student, 1985; **National Alexandre Joel Prize for young investigator,** 1995 (Association for Cancer Research, ARC, France); **National Prize for Fundamental Cancer Research/young investigator,** 1995 (French Society of Cancer and National Federation of Cancer Institutes, France); **Lawrence Berkeley National Laboratory Outstanding Performance Award,** 1998 (Lawrence Berkeley National Laboratory, Berkeley, CA); **Integrated Science Partnership Program Appreciation Award,** 1999 (Lawrence Berkeley National Laboratory, Berkeley, CA)

Teaching Fellowship: **University of Paris,** 1991-1994;

Research Fellowships: **French Ministry of Education and Research** (France), graduate fellowship, 1991-1994; **International Agency for Research on Cancer** (IARC-WHO), postdoctoral fellowship, 1995-1996; **Association for Cancer Research** (ARC), complementary fellowship, 1996; **Department Of Defense**/USA-Breast Cancer Research Program (Postdoctoral Training grant) 1997-1999

Collaborative Research Fellowships: **French Society of Cancer Travel Fellowship,** 1995; **Journal of Cell Science Travel Fellowship,** 1997; **Philippe Foundation Travel Fellowship,** 1998.

Chair at scientific meetings: Session on "Cellular Organization, Signal Transduction and Cancer" at the "Biology and Mathematics of Cells: Physiology, Kinetics and Evolution" ESMTB meeting, Spain, 2001; co-organizer and session co-chair on "nuclear compartmentalization in differentiation and cancer" at the International Society of Differentiation meeting, France, 2002.

Guest Scientist: Lawrence Berkeley National Laboratory (2000-ongoing)

PATENTS

US 6,287,790 B1: *"Utilization of nuclear structural proteins for targeted therapy and detection of proliferative and differentiation disorders,"* S.A. Lelièvre and M.J. Bissell, 2001.

INVITED SEMINARS AND LECTURES

Speaker at national and international meetings:

"The solid-state signaling pathway from the extracellular matrix to the nuclear matrix: the critical role of 3D

architecture at the cellular level", High resolution X-ray CMT Workshop (LBNL, Berkeley, CA), August 1996; **"Internal cell architecture-A new look"**, Advanced Light Source Users Meeting (LBNL, Berkeley, CA), October 1997; **"Global rearrangement of nuclear matrix-associated proteins when human mammary epithelial cells are cultured in 3-D: an analysis using confocal-, electron-, and soft x-ray microscopy"**, Keystone Symposium on Nuclear Matrix (Copper Mountain, CO; junior investigators workshop), April 1998; **"Nuclear structure, cell proliferation, and tissue morphogenesis"**, American Society for Cell Biology Meeting (San Francisco, CA), December 1998; **"Tissue architecture and gene expression: study of tissue matrix in three-dimensional models of cell culture"** and **"The non-chromatin structure of the nucleus or nuclear matrix: study of its interaction with the chromatin structure and its role in the regulation of gene expression"**, Biomathematics Summer School (Termoli, Italy), Mathematics in Cell Physiology and Proliferation, June 1999; **"Nuclear-directed signaling in mammary gland acini"**, Gordon Conference on Biological Structure and Gene Expression (Meriden, NH; short talk), August 1999; **"Nuclear organization in normal and malignant breast: NuMA is a marker of cell phenotype and a regulator of differentiation"**, Era of Hope DOD Breast Cancer Research Meeting (Atlanta, GA; platform talk), June 2000; **"Cell cycle regulation in higher order cell assemblies: the role of three-dimensional tissue architecture,"** Third International Congress of Nonlinear Analysts (Catania, Sicily) July 2000; **"Signal transduction and feedback signaling ", "Cellular transformation and genomic instability", "Tumor progression: How in vitro models may help understand in vivo situations"**, ESMTB School, Biology and Mathematics of Cells: Physiology, Kinetics and Evolution, (Siguenza, Spain) June 2001; **"NuMA functionally links cell adhesion and nuclear structure to regulate cell survival in breast"**, 2nd International Conference on Tumor Microenvironment: Progression, Therapy and Prevention, (Baden, Austria), June 2002; **"Cellular interactions in differentiation and cancer with emphasis on cell-extracellular matrix interactions", "Extracellular matrix signaling to chromatin", "Chromatin remodeling in cancer"**, International School "Using Mathematical Modeling and Computer Simulation to Improve Cancer Therapy" (Propriano, Corsica), September 2002; **"Multiple facets of nuclear structural proteins: The Role of NuMA is the regulation of breast epithelial phenotypes"**, 12th Internatioanl Conference of the International Society of Differentiation, (Lyon, France), September 2002.

Seminars:

"The nuclear matrix is an old concept still in its infancy", Gustave Roussy Cancer Institute, Villejuif, France (Dept. of Clinical and Molecular Pharmacology), September 1995; **"The solid-state pathway: a model for the regulation of gene expression"**, University of Paris XII, Creteil, France (CRRET Laboratory), June 1996; **"From the extracellular matrix to the nuclear matrix, the dynamic cellular architecture plays a role in the regulation of cellular behavior: a study of a model of mammary tumorigenesis"** Gustave Roussy Cancer Institute, Villejuif, France (Dept. of Clinical and Molecular Pharmacology), June 1996; **"Dynamic re-organization of nuclear architecture during tumorigenesis and tumor reversion"**, Harvard Children's Hospital, Boston (Dept. of Dr. Judah Folkman), April 1997; **"The role of cellular and tissue structure during tumorigenesis"**, Institute of Immunology, Munich, Germany (Dept. of Dr. G. Riethmüller), June 1997; **"Dynamic reciprocity between the extracellular matrix and the organization of the cell nucleus: a study of mammary epithelial cell morphogenesis"**, Institut de Génétique Moléculaire, Paris, France, June 1998; **"Interrelationships between the distribution of nuclear matrix proteins, chromatin structure and gene expression during mammary epithelial cells morphogenesis"**, CEA, Fontenay aux Roses, France, June 1998; **"Communication between the extracellular matrix and the nuclear structure in breast development and malignancy"**, Boston University Medical School, Dept of Biochemistry, February 1999; **"The role of nuclear organization in normal and malignant breast structures"**, California Pacific Medical Center Research Institute (San Francisco, CA), May 1999; **"Nuclear organization in normal and malignant breast"**, Division of Radiation and Cancer Biology, New England Medical Center, TUFTS University, Boston, MA); **"What is the link between nuclear architecture and the expression of malignancy?"**, Purdue University, Dept. of Basic Medical Sciences (West Lafayette, IN), March 2000; **"The organization of the cell nucleus in breast differentiation and tumorigenesis. A source for the development of novel anticancer strategies,"** Research Institute of MolecularPathology, Vienna Biocenter (Boehringer-Ingelheim,Vienna, Austria), April 2000; **"Structure, Instability and Plasticity in Cancer"** University of Mexico, Mexico City, September 2001; **"Subcompartmentalization of Nuclear Proteins in Differentiation and Cancer: Multi-faceted NuMA Regulates Breast Epithelial Cell Behavior."** IUPUI, Indianapolis, IN, November 2001.

PUBLICATIONS

K Bojanowski, **S Lelièvre**, J Markovits, J Couprie, A Jacquemin-Sablon and AK Larsen, "Suramin is an inhibitor of DNA topoisomerase II *in vitro* and in Chinese hamster fibrosarcoma cells". *Proc.Natl.Acad.Sci., USA*, 89:3025-3029, 1992.

S Lelièvre and AK Larsen, "Development and characterization of suramin-resistant Chinese hamster fibrosarcoma cells: drug-dependent formation of multicellular spheroids and a greatly enhanced metastatic potential." *Cancer Res.*, 54: 3993-3997, 1994.

S Lelièvre and AK Larsen, "L'acquisition de la résistance à la suramine dans les cellules de fibrosarcome de hamster chinois s'accompagne de modifications morphologiques et d'une augmentation de leurs capacités métastatiques". *Bull. Cancer*, 81: 903-905, 1994.

S Lelièvre and AK Larsen, "Chronic in vitro suramin exposure leads to the development of drug resistant sublines which grow as three dimensional cultures and are highly invasive *in vivo*. Lack of growth factor involvement in the cytotoxic action of the drug." *In* "Novel approaches in anticancer drug design. Molecular modelling-New treatment strategies. *Contrib. Oncol.*, 49: 117-123, 1995, (WJ Zeller, D'Incalci M, and Newell DR, eds), Basel, Karger.

S Lelièvre, Y Benchokroun, and AK Larsen, "Altered DNA topoisomerase I and II in suramin-resistant Chinese hamster fibrosarcoma cells." *Mol. Pharmacol.*, 47: 898-906, 1995.

S Lelièvre, VM Weaver, and MJ Bissell, "Extracellular matrix signaling from the cellular membrane skeleton to the nuclear skeleton: a model of gene regulation in mammary epithelial cells." *Recent Progress in Hormone Research*, 51:417-432, 1996.

S Lelièvre and Mina J. Bissell. "The solid-state signaling pathway from extracellular matrix to nuclear matrix: the critical role of three-dimensional architecture for functional differentiation." Proceedings of the 1996 Workshop on High Resolution Computed Microtomography (CMT), LBNL/UC, pp 85-96, 1997.

S Lelièvre, VM Weaver, CA Larabell, and MJ Bissell, "Extracellular matrix and nuclear matrix interactions may regulate apoptosis and tissue-specific gene expression: a concept whose time has come." *In* Advances in Molecular and Cell Biology: Cell Structure and Signaling, (RH Getzenberg, ed), JAI Press Inc, Greenwich CT, Vol 24, pp: 1-55, 1997.

SA Lelièvre, VM Weaver, JA Nickerson, CA Larabell, A Bhaumik, OW Petersen, and MJ Bissell. "Tissue phenotype depends on reciprocal interactions between extracellular matrix and the structural organization of the nucleus" *Proc. Natl. Acad. Sci. (USA)*, 95: 14711-14716, 1998.

SA Lelièvre and MJ Bissell. "Communication between the cell membrane and the nucleus: the role of protein compartmentalization" 25th Anniversary Issue of *J. Cell. Biochem,* 30/31 suppl.: 250-263, 1998

MJ Bissell, VM Weaver, **SA Lelièvre**, F Wang, OW Petersen, and KL Schmeichel, "Tissue structure, nuclear organization and gene expression in normal and malignant breast" *Cancer Res.*(SUPPL), 59: 1757s-1764s, 1999.

H-M Chen, KL Schmeichel, IS. Mian, **SA Lelièvre**, OW Petersen, and MJ Bissell. AZU-1: a candidate breast tumor suppressor and biomarker for tumorigenic reversion. *Mol.Biol.Cell*, 11: 1357-1367, 2000.

SA Lelièvre, P. Pujuguet, and MJ Bissell. "Cell nucleus in context." *Crit. Rev. Eukar. Gene Expression*, 10: 13-20, 2000.

W Meyer-Ilse, D Hamamoto, A Nair, **SA. LeLièvre**, G Denbeaux, L Johnson, A Lucero, D Yager, and CA. Larabell. "High Resolution Protein Localization Using Soft X-ray Microscopy." *J. Microscopy,* 201: 395-403,2001.

C Ortiz de Solorzano, R. Malladi, **SA Lelièvre**, and SJ Lockett. "Segmentation of nuclei and cells using membrane related protein markers." *J. Microscopy* , 201: 404-15, 2001.

SK Muthuswamy, D Li, **SA Lelièvre**, MJ Bissell, and J Brugge. " ErbB2, but not ErbB1, can reinitiate proliferation and induce luminal repopulation in growth-arrested epithelial acini" *Nature Cell Biology,* 3: 785-792, 2001.

VM Weaver, **SA Lelièvre**, JN Lakins, J Jones, F Giancotti, Z. Werb, and MJ Bissell. "Beta4-integrins induce polarity and apoptosis-resistance in mammary tissue structures". (In revision for Cancer Cell)

SA Lelièvre, VM Weaver, CA. Larabell, and MJ Bissell. "Inside-out signaling by NuMA connects basement membrane signaling and apoptosis". (Submitted)

C Plachot and **SA Lelièvre**. New directions in cancer biology: from basement membrane-directed polarity to DNA methylation. Mathematical Biology and Medicine Series, "Cancer modeling and simulation", Chapman & Hall/CRC (In preparation).

P Abad, A Viron, JA Nickerson, E Puvion, and **SA Lelièvre**. NuMA shuttles between different subcellular locations in differentiated and tumor breast epithelial cells. (In preparation).